sample. In all cases duplicates represent separate extractions.

Sample	Per cent moisture	Per cent ash	Per cent indigotin
Purified synthetic indigo	....	....	99.71
	....	....	100.59
Awa indigo No. 1	9.71	27.75	6.78
	9.41	27.41	6.86
Awa indigo No. 2	9.18	27.28	5.05
	9.30	27.17	5.15
Awa indigo No. 3	10.08	30.56	4.23
	10.10	30.47	4.32
Awa indigo No. 4	12.44	32.39	1.94
	12.44	32.48	2.02
Awa indigo No. 5	9.24	33.89	1.26
	9.21	33.77	1.34
Awa indigo No. 6	11.56	34.50	2.46
	11.56	34.55	2.47
Awa indigo No. 7	9.92	34.34	3.17
	9.95	34.32	3.23
Awa indigo No. 8	9.46	35.26	3.59
	9.38	35.08	3.72
Indigo by Nagai process	3.18	33.25	31.45
	3.28	33.25	31.53

State College of Washington
Pullman, Washington

EXPERIMENTAL DATA COMPARING THE DELICACY OF DIFFERENT TESTS FOR HYDROGEN PEROXIDE IN MILK

By Irwin T. Darlington

Received April 12, 1915

From references on the detection of hydrogen peroxide, the reagents listed in Table I were found to be in most general use and appear to give the most delicate tests for the detection of hydrogen peroxide in milk.

A stock solution of hydrogen peroxide was made from a commercial sample containing 3.2 per cent of hydrogen peroxide by analysis with potassium permanganate. This stock solution was made so that each cc. contained 0.01 g. of hydrogen peroxide.

Table I

Reagent	Solution	Color in presence of H_2O_2
Starch	Dilute (water) + KI	Bluish green
Paraphenylene diamine	2 per cent (water)	Blue
Benzidine	4 per cent (alcoholic) + 10 cc. milk (+ 2 or 3 drops acetic acid)	Blue
Titanic acid	2 per cent solution titanium hydrate in dilute H_2SO_4	Yellow
Vanadic acid	2 per cent in dilute H_2SO_4	Faint orange-red

The milk to which the hydrogen peroxide was added was received from the city inspectors and was proven free from hydrogen peroxide by each of the above reagents.

Hydrogen peroxide was added to each 100 cc. of milk in separate bottles to the amounts indicated in Table II, and tests made on 10 cc. of each sample with each of the above reagents. Tests were made immediately after the samples were made up and again after standing 18 hours in a warm room.

Table II

	Results of tests made immediately after samples were made up																	After standing 18 hrs. in a warm room		
Grams per 100 cc.	15.0	9.0	3.0	1.5	0.75	0.6	0.3	0.15	0.03	0.015	0.006	0.003	0.006	0.003	0.00105	0.00075	0.00037	15.0	9.0	3.0
Starch	+	+	+	+	+	+	+	+	+	+	+	+	+	+	—	—	—	+	—	—
Paraphenylene	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	—	+	+	—
Benzidine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	—	+	—	—
Titanic acid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	—	—	—	+	—	—
Vanadic acid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	—	—	—	+	—	—

It will be seen from Table II that paraphenylene diamine and benzidine proved to give the most delicate tests for hydrogen peroxide in raw milk. The limits of these two reagents proved to be 0.00075 g. of hydrogen peroxide in 100 cc. of raw milk. No experiments were made with milk that had been previously heated to destroy bacterial action.

Practically all the hydrogen peroxide was reduced after standing 18 hours in a warm room. This confirmed previous results obtained in this laboratory when it was found that after 18 hours' standing at room temperature no reaction could be obtained from the addition of 1 g. of magnesium peroxide to 100 cc. of raw milk. Positive tests were given only when the milk contained 9 per cent and 15 per cent of hydrogen peroxide.

The samples containing 9 per cent and 15 per cent of hydrogen peroxide failed to give a positive test with any of the above reagents, except paraphenylene diamine, which gave a delicate positive test with 15 per cent of hydrogen peroxide after standing in a warm room for 24 hours. Tests on distillates of milk treated with peroxide were negative.

A few tests were made with magnesium dioxide added as a preservative. The experiments made with this material were insufficient to afford any positive conclusions, but it appears that magnesium dioxide does not lend itself to as delicate tests as does hydrogen peroxide.

From the foregoing it is seen that paraphenylene diamine is the test of the reagents tested for the detection of small quantities of hydrogen peroxide in raw milk, and that it is probable that peroxide in amounts that would be added to milk as a preservative cannot be detected after 18 hours by these reagents.

Chemical Laboratory, Department of Health
City of New York

THE OFFICIAL METHOD FOR DETERMINING CRUDE FIBER AS APPLIED TO COTTONSEED MEAL

By C. K. Francis

Received April 7, 1915

The well known Weende method[1] for determining crude fiber has been subjected to criticism by many chemists. The chief trouble seems to be with the filtering materials, linen, asbestos, or glasswool, which vary in their physical characteristics and, necessarily, in filtering efficiency. In a number of laboratories it has been the practice to use linen cloth for filtering. The cloth has been used in the same manner as filter paper, with and without suction, in plain and in ribbed funnels. It has been difficult to duplicate linen of a selected pick, so that the fineness of the filter has been liable to vary from time to time. However, the method seemed to be satisfactory when working with such substances as corn, but when one endeavored to filter the alkaline mixture of certain seeds like flaxseed and cottonseed it became necessary to devise some scheme to hasten the process.

A novel arrangement for filtering the mixture through cloth by upward suction has been suggested by Pickel.[2] The cloth stretched over a funnel or thistle-tube furnishes a flat surface and the liquid passes rapidly, but the method appears to give results which are too low. Forbes and Menching[1] have proposed a method in which fine sand is used as a filter, but our tests with this material gave no results because the cottonseed meal mixture did not pass through even after 24 hours' continuous suction. Sweeney[2] has proposed to omit the first filtration and neutralize the acid with a known amount of sodium hydroxide, then to proceed with the alkaline treatment in the same vessel, and filter through asbestos. The method has been modified in a slight detail by Kennedy[3] and the procedure has been tested by several chemists in this laboratory. The results obtained for cottonseed meal were somewhat higher than when two filtrations were made.

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[1] "Official Methods of Analysis," U. S. Bureau of Chemistry, *Bull.* **107** (Rev.), 65.

[2] This Journal, **2** (1910), 280; see MacNider, *Ibid.*, 281.

While studying the methods for determining crude fiber our attention has been centered upon the filtering materials, and we have endeavored to select a filter which would be uniform. Slight modifications are suggested, and the work of several investigators, especially that of Sweeney, is taken into consideration in the directions given below. The specific details mentioned, it is believed, do not alter the principles involved in the official method, but on the other hand, suggest a procedure which permits a greater accuracy than the directions usually followed.

EXTRACTION OF FAT—Two grams of the meal are weighed into a glass extraction tube provided with a plug (about an inch square) of fat-free absorbent cotton in the bottom. More cotton is placed above the sample and a small copper wire spring inserted to hold the charge in place. After drying six hours at 105°, the meal is placed in the extractor[4] and extracted with dry ether for 16 hours. The excess ether is removed by suction, the tube and contents dried in the electric oven at 105°. The loss in weight is calculated to per cent of crude fat or oil.

CRUDE FIBER METHOD—The dry fat-free residue is carefully brushed, over white glazed paper, from the glass extraction tube into an 800 cc. lipless beaker, care being taken to remove any particles of the meal that may cling to the cotton plugs. Two hundred cc. of boiling 1.25 per cent H_2SO_4 are added and the mixture boiled for 30 minutes, using as condensers round bottom flasks filled with cold water on top of the beakers as shown in Fig. I: the flasks will fit closely if the beakers are round and have well-formed rims. A blast of air should be directed into the beaker when the foaming becomes serious. At the end of 30 minutes, 200 cc. of boiling 3.52 per cent NaOH are added and the boiling continued another 30 minutes.

Sweeney and others have proposed a sodium hydroxide solution of another strength but have included a separate neutralization, in the presence of an indicator, with a 10 per cent NaOH solution. A number of tests have indicated that a 3.52 per cent solution will neutralize the acid and leave a 1.25 per cent sodium hydroxide solution. Numerous titrations of this liquid were made showing the strength to vary from 1.25 to 1.27 per cent NaOH. According to the procedure outlined, one filtration is saved and there is no need to add an indicator and carefully neutralize.

A Buchner funnel, outside diameter 10.5 cm., is prepared with a 9 cm. S & S 575 filter paper. The paper should be moistened with water and pressed well into place, so that there are no holes uncovered and no open channels left about the edges. It is advisable to place a glass filter plate over the paper, to protect it and to prevent excessive packing of the fiber.

After the 30 minutes' boiling, the fiber should be filtered off rapidly with the aid of suction, washing with boiling water and then with a 1.25 per cent solution of HCl[1] (HCl 14 cc. made up to 500 cc.) until the washings are acid. Much time may be saved if the liquid is set aside for about five minutes to allow the fine material to settle so that the clear liquid may be decanted. When this has been done it has not, so far as we were able to determine, influenced the final results. The fiber should be washed with hot water until free from chlorides, and finally the filter should be washed several times with alcohol. The residue on the paper will be almost free from water and may be transferred to a prepared[2] Gooch crucible with the aid of a spatula and a small amount of 80 per cent alcohol.

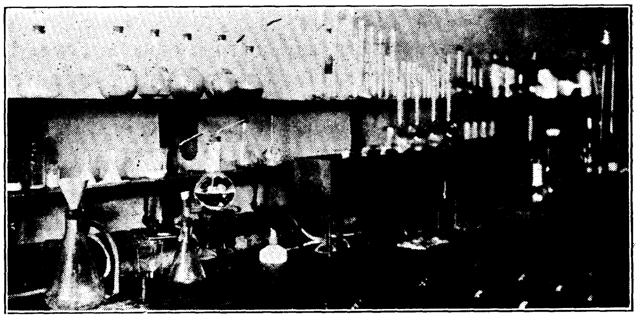

FIG. 1—CRUDE FIBER APPARATUS

The crucible is placed in an oven heated to 105–110° C. for 2 to 6 hours, cooled and weighed. Materials containing much fiber may require heating for the longer period, and a second drying and weighing may be necessary in order to get the weight constant, but many determinations show a constant weight after heating for 2 hours. When dry the crucible is partially covered and incinerated at a low red heat for 15 minutes or until a white or light gray ash is obtained; it is then cooled and weighed. The loss in weight may be calculated as crude fiber.

EXPERIMENTAL

The tests described in the first part of this paper were made on the same sample of cottonseed meal. The quantity weighed for analysis was two grams, unless otherwise stated. All results are in terms of crude fiber calculated to per cent of the original material. The composition (except fiber) of the meal used in the experiments is shown in Table I.

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[1] THIS JOURNAL, **5** (1913), 258; Ohio Agricultural Experiment Station, *Bull.* **255** (1913).

[2] U. S. Bureau of Chemistry, *Bull.* **137** (1910), 157.

[3] THIS JOURNAL, **4** (1912), 600.

[4] Francis, *Ibid.*, **3** (1911), 673.

[1] C. Kennedy, *Loc. cit.*, uses H_2SO_4 here but we prefer HCl, because the chlorides are easily detected and removed; moreover, dilute solutions of HCl may be quickly prepared.

[2] A porcelain or platinum Gooch crucible may be used. The crucible is prepared with a pad of asbestos, about 1 mm. thick, and heated to redness for fifteen minutes. When cool, fit the crucible into a suction filtering tube and transfer the material to it.

TABLE I—PERCENTAGE COMPOSITION OF MEAL

Moisture	Ash	Crude protein	Crude fat
9.32	5.09	45.44	7.07

TESTING THE ASBESTOS—A test was made to determine the effect of digesting the asbestos, used for filtering, in acid and alkali and igniting before use. Portions of the same sample were treated alike with the exception that one lot was filtered through prepared asbestos[1] while the untreated asbestos was used for the second lot. The results with the untreated asbestos were 1.3 per cent higher than those obtained with the prepared asbestos.

It appeared that the crude fiber should be completely burned at comparatively low temperatures, and that extra heating in the flame of the blast burner should not be necessary. In Table II the results of a test are shown in which quantities, 1.3 grams to 2.4 grams, of prepared asbestos were ignited to a constant weight and then placed in the blast flame for 3 minutes. This asbestos was used in blank determinations and consequently, subjected to action of the acid and alkali.

TABLE II—LOSS DUE TO EXCESSIVE HEATING OF THE ASBESTOS

Crucible No.	Wt. cr. + asbestos ignited over Bunsen flame for 3 hrs.	5 hrs.	Loss Gram	Ignited 3 min. in blast flame	Loss Gram
22	17.8641	17.8616	0.0035	17.8553	0.0063
21	17.6653	17.6645	0.0008	17.6575	0.0070
24	17.1209	17.1191	0.0018	17.1098	0.0094
19	18.0400	18.0395	0.0005	18.0310	0.0085
10	17.8555	17.8548	0.0007	17.8479	0.0069
16	17.7495	17.7487	0.0008	17.7400	0.0087

A separate test of the prepared asbestos was made to determine the loss due to heat alone, that is, without having to consider any influence the reagents might have on the final loss. For this purpose portions weighing from 0.5 to 2.2 grams were dried to constant weight and then placed in the blast flame for 3 minutes. The loss was from 8.8 to 33.2 mg., respectively, or a percentage loss of 1.85, 1.64 and 1.49 when calculated on a basis of the weight of the asbestos. When calculated as crude fiber, this loss represents 0.44, 0.76 and 1.66 per cent.

TABLE III—CRUDE FIBER IN COTTONSEED MEAL AS DETERMINED BY THE ONE-FILTRATION METHOD AND DIFFERENT FILTERS

Kind of filter	Per Cent Crude Fiber Determinations			Av.	Remarks
Linen	8.57	8.58	8.54	8.56	No suction; res. washed into a Gooch.
Hard paper	9.54	9.53	9.37	9.48	S & S 575, 9 cm. in Buchner funnel.
Asbestos	10.87	10.81		10.84	Filter in a Hirsch funnel.

The fibrous material present in feeds is of such a nature that it should be completely incinerated after a few minutes (say 15) in the flame of a good burner, and ignition at high temperatures will tend to increase the loss which is calculated as crude fiber. In the fourth column of Table II, the weights are shown to be constant, excepting the material in crucible No. 22. Further loss, shown in the last column, obtained in the blast flame is probably due to some action on the asbestos or volatile salts present and certainly not anything closely related to cellulose or fiber.

TABLE IV—CRUDE FIBER IN COTTONSEED MEAL AS DETERMINED BY THE TWO-FILTRATION METHOD AND DIFFERENT FILTERS

Kind of filter 1—After acid digestion	2—After alkali digestion	Per Cent Crude Fiber Determinations			Av.	Remarks
Linen	Linen	8.44	8.16	7.96	8.19	No suction; residue washed into a Gooch crucible.
Linen	Asbestos	8.56	8.82		8.69	Asbestos filter in a Gooch crucible, *i. e.*, small amount asbestos used.
Linen	Asbestos	8.96	8.79	9.02	8.92	Asbestos filter in a Hirsch funnel, *i. e.*, large amount asbestos.
Paper	Paper	9.63	9.53	9.51	9.56	S & S 575 paper in a Buchner funnel.

FILTERING MATERIALS—The data presented in Table III show, to some extent, the loss that may take place with different kinds of filters when but one filtration is made.

Every precaution was taken in each test to wash free from alkali, acid and chlorides. The linen used was of good grade, having 38 threads to the sq. cm. The paper was hard filter paper, S & S No. 575, diameter 9 cm.

In Table IV are shown results obtained with the same sample of cottonseed meal, when two filtrations were made. For this experiment the regular 1.25 per cent acid and alkaline solutions were used according to the official methods.

It will be observed from a study of the data in Tables III and IV, that the determinations with a given filter appear, when considered alone, to be satisfactory. When comparisons of the groups are made, it will be seen that the results do not agree.

Determinations in addition to those reported here have shown that the loss with linen depends upon the number of times the mixture is filtered through it, thus demonstrating that there is a loss of crude fiber. The high results in Table III with asbestos have been explained under Table II, as being caused by the asbestos losing weight during the ignition process, and that the loss fluctuates with the quantity of asbestos used.

The crude fiber determinations in which filter papers were used appear to be the most uniform for both methods as indicated in Tables III and IV. The paper filters certainly do not permit any solid matter to pass into the filtrate, and they offer a uniform filter which may be easily obtained and from which the residue can be readily transferred.

Some determinations were made in which asbestos was used for both filtrations according to the official method, but the large quantity of material added to the second filter in this way caused the filtration to proceed very slowly, and introduced an additional error which may have resulted from any loss of weight of the asbestos.

PROTEIN REMOVED—An analysis of the residue remaining on the filter paper after the acid digestion, showed a protein content of 5.75 per cent. The final residue, chiefly crude fiber, contained 0.38 per cent protein. The crude fiber extracted from another meal was found to contain 0.35 per cent protein. This amount confirms the results obtained by Cross and Bevan, Tollens and others,[1] when working with the ordinary methods for determining crude fiber. No doubt any good filter will retain approximately the same quantity of protein, pentosans and volatile ash known to be present in crude fiber.

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[1] Asbestos which had been digested in HNO_3, NaOH and H_2O; also ignited in a furnace. This asbestos was used wherever asbestos is mentioned.

[1] *Cellulose*, **1** (1895), 101.

TESTS ON CELLULOSE—Two samples of filter paper were prepared from S & S paper No. *597*. One sample, designated *coarse paper*, consisted of large pieces, each about the size of one-quarter of a 9 cm. paper. All samples for the coarse paper work were dried at 105° C., until the weight became constant. The second sample, here called *fine paper*, was made from the same kind of paper ground so as to pass a 40-mesh sieve. Both samples were boiled in the acid and alkaline solutions so as to determine the action of the reagents. In Tables V and VI the procedure is indicated. The words, *acid only*, serve to show that the portions were boiled in 1.25 per cent sulfuric acid for one-half hour, then filtered through paper, washed, dried, weighed, ignited and weighed. The treatment with alkali was similar except that 1.25 sodium hydroxide was used. The digestions in both reagents were made as outlined in the method suggested when but one filtration is to be used, and, also, according to the directions of the official methods, which require two filtrations.

TABLE V—ACTION OF CRUDE FIBER REAGENTS ON PURE FILTER PAPER
Coarse Paper, Not Pulverized

On Paper Filters				On Asbestos Filters			
	Fiber Recovered				Fiber Recovered		
		Per cent on				Per cent on	
Weight of sample Gram	Wt. in gram	Sample basis	Ash + H_2O free basis	Weight of sample Gram	Wt. in gram	Sample basis	Ash + H_2O free basis
ACID DIGESTION ONLY				ACI D DIGESTION ONLY			
0.6333	0.6230	98.37		0.5766	0.5705	98.94	
0.5551	0.5496	99.01		0.6229	0.6315	101.38	
0.6084	0.5967	98.08		0.5506	0.5589	101.51	
	Average,	98.49	99.31		Average,	100.61	101.44
ALKALI DIGESTION ONLY				ALKALI DIGESTION ONLY			
0.6250	0.6018	96.29		0.5579	0.5531	99.13	
0.5903	0.5664	95.95		0.5456	0.5386	98.72	
0.5829	0.5558	95.35		0.5390	0.5304	98.40	
	Average,	95.86	96.66		Average,	98.75	99.58
ACID AND ALKALI				ACID AND ALKALI			
0.5227	0.4595	87.95		0.5493	0.4863	88.53	
0.5657	0.4955	87.59		0.5271	0.4667	88.54	
0.5569	0.4867	87.39		0.5535	0.4902	88.56	
	Average,	87.64	88.36		Average,	88.54	89.28

TABLE VI—ACTION OF CRUDE FIBER REAGENTS ON PURE FILTER PAPER
Fine Paper, Pulverized to Pass 40-Mesh Sieve

On Paper Filters				On Asbestos Filters			
	Fiber Recovered				Fiber Recovered		
		Per cent on				Per cent on	
Weight of sample Gram	Wt. in gram	Sample basis	Ash + H_2O free basis	Weight of sample Gram	Wt. in gram	Sample basis	Ash + H_2O free basis
(1) ACID DIGESTION ONLY				(*a*) ACID DIGESTION ONLY			
0.3130	0.2939	93.90	99.42	0.2166	0.2191	101.15	107.14
0.1528	0.1452	95.03	100.62	0.2091	0.2144	102.53	108.56
0.1821	0.1722	94.56	100.11	0.2288	0.2228	97.38	103.10
	Average,	94.49	100.05		Average,	100.35	106.27
(2) ALKALI DIGESTION ONLY				2(*a*) ALKALI DIGESTION ONLY			
0.2180	0.1991	91.33	96.70	0.2112	0.2071	98.06	103.86
0.1630	0.1504	92.27	97.72	0.1955	0.1927	98.52	104.38
0.1388	0.1274	91.78	97.17	0.2145	0.2118	98.74	104.59
	Average,	91.79	97.20		Average,	98.44	104.28
(3) ACID AND ALKALI: 1 FILTRATION				3(*a*) ACID AND ALKALI: 1 FILTRATION			
0.1485	0.0964	64.91	68.75	0.1993	0.1425	71.50	75.71
0.2321	0.1501	64.67	68.47	0.1934	0.1445	74.71	79.13
	Average,	64.79	68.61	0.2193	0.1601	73.00	77.30
					Average,	73.07	77.38
(4) ACID AND ALKALI: 2 FILTRATION							
0.4437	0.2885	65.02	68.85				
0.4082	0.2667	65.58	69.18				
0.4432	0.2848	64.26	68.05				
	Average,	64.95	68.69				

When considered alone, the action of the acid on the coarse paper is very slight and the alkali does not seem to dissolve a very large quantity, but when the acid digestion is followed by an alkaline digestion the loss is very material. The total loss, shown in Table V, through the paper filter is 11.64 per cent; the loss through asbestos is not so large, 10.72 per cent. In each test with asbestos filters the amount of fiber recovered appeared to be larger than with the paper filters, but it has been demonstrated that there is a compensating error here on account of the asbestos losing weight.

The results with tests on fine paper are given in Table VI.

Digestions of the fine paper in alkali, and in both acid and alkali resulted in greater losses than those of coarse paper. These experiments confirm the results shown in Table V regarding the influence of the treatment in acid (1) on the subsequent action of the alkali (3). There is practically no loss in acid, but a small loss in alkali (2), yet a large loss when the material is acted on by both acid and alkali, (3) and (4).

The quantity of fine paper recovered after digestion in alkali only, or in acid and alkali was much less than when the coarse paper was used. This indicates that samples should always be reduced to a fair degree of fineness.

Tests (3), (4) and 3(*a*), Table VI, are supposed to give the same results. The good checks obtained for (3) and (4) show that the one-filtration method will give equally as good results as the regular two-filtration method.

As cottonseed meal contains a quantity of lint, corresponding to fine paper, it may be fair to assume that the actual amount of crude fiber in the ordinary meal is much larger than will be found by the official crude fiber method. Suppose the results with paper filters in Table III and those in Table IV are correct, giving about 9.5 per cent crude fiber, then this amount represents 68.6 per cent of the total quantity of fiber present—see Table VI, (3) and (4). By simple calculation the total fiber becomes 13.8 per cent, and, therefore, 4.3 per cent is soluble or digestible.

SUMMARY AND CONCLUSIONS

1—A method is proposed for determining crude fiber in feedstuffs in which filter paper is to be used as the filtering medium, and which requires but one filtration.

2—Loss of crude fiber may occur when linen is used as a filter.

3—Asbestos may lose weight and thus introduce errors, causing the results to be too high. The error depends upon the weight of asbestos used.

4—The solvent action of 1.25 per cent sulfuric acid on practically pure fiber (filter paper) is negligible.

5—The treatment in sulfuric acid has an influence on the subsequent action of the alkali.

6—Coarse paper dissolves to the extent of 3.44 per cent during the 30 minutes' boiling in 1.25 $NaOH$, while fine paper dissolves to the extent of 2.8 per cent.

7—When filter paper is boiled in both acid and alkali, the loss with coarse paper is 11.64 per cent and with fine paper 31.4 per cent.

8—The proposed one-filtration method, with hard filter paper, gives more reliable results than those commonly obtained by the present official method for crude fiber.

Oklahoma Agricultural and Mechanical College
Stillwater, Oklahoma

THE APPARENT EFFECT OF ACETIC ACID UPON THE CONSTANTS OF BUTTER FAT

By Clarence Bahlman

Received April 23, 1915

During the course of a chemical and bacteriological examination of Cincinnati's ice cream supply carried on by the laboratory of the Cincinnati Department of Health,[1] it became necessary to examine each sample for percentage of milk fat as well as to determine the presence or absence of foreign fats and oils.

The careful attention required for the estimation of fat by the laborious Roese-Gottlieb method would have made it impossible to examine more than a few samples each day. By the use of a simpler method, the laboratory helper was enabled to arrive at the fat content of 20 or 30 samples within one hour's time.

By this method,[2] 9 grams of the melted ice cream are weighed into the ordinary 10 per cent Babcock milk bottle, 20 cc. of glacial acetic acid added, and the mixture thoroughly shaken: 10 cc. of Babcock sulfuric acid are then added and the bottle again shaken. From this point the sample is treated by the ordinary Babcock method for the estimation of fat in milk. The reading of the fat column in the neck of the bottle, multiplied by two, gives the percentage of butter fat.

With this technique, the charring effect of the sulfuric acid upon the sugar is eliminated. The separated fat has a clear, pale yellow color, is free from sediment and foam, and distinctly separated from the lower acid layer.

As a test for foreign fats and oils, a few drops of the clear fat from the Babcock bottles were placed in the Zeiss butyro-refractometer and the refractive index determined at 40° C. With this instrument, pure fat from cows' milk will give, at 40° C., a reading falling between 40.5 and 44.5 scale divisions.

Practically all of the readings upon the samples, however, were well below the minimum figures for pure butter fat. As a general rule, the readings varied from 38.0 to 41.0, with a few running as low as 36.5 scale divisions.

Since it appeared highly improbable that all of our ice cream manufacturers were practicing adulteration, and in view of the fact that the food chemist places such great reliance upon the refractometer, the abnormal results shown by the samples were the subject of further study.

EXPERIMENTAL

One of the most suspicious samples was selected and about 15 grams of fat isolated, both by ether extraction and by the acetic acid Babcock method. The fat obtained by the latter method was taken from the necks of the bottles with a pipette and filtered through paper.

The fat separated by ether extraction gave results for refractive index and other constants which agreed with those for a pure butter fat. The fat obtained by the acetic acid method, however, gave low readings for refraction value and decidedly high Koettstorfer and Reichert-Meissl numbers, although a normal Hanus iodine figure was obtained.

It would appear from the above that when butter fat is separated from dairy products in the presence of acetic acid, the constants are materially altered. The following experiments were undertaken to determine whether such abnormalities were to be attributed to complex changes or merely to mechanical retention of the acid.

EXPERIMENT I—Nine grams of rich cream of known purity were placed into each of 15 Babcock bottles and 20 cc. of glacial acetic acid added. After mixing, 10 cc. of Babcock sulfuric acid were added and the bottles centrifuged as usual. The separated fats were drawn off, combined, and filtered through paper.

Fat was also separated from the same cream by the usual Babcock method of adding 17.5 cc. of sulfuric acid to 18 grams of cream.

After standing in the refrigerator over night, the well-known constants for butter fat were determined, with results shown in Table I.

Table I

Fat obtained by	Babcock method	Acetic acid method	
Refractometer reading, 40° C	41.8	39.4	
Hanus iodine number	28.9	29.1	
Koettstorfer number	227.0	242.0	[226.0]
Reichert-Meissl number	28.3	44.4	[27.2]
Soluble acids	6.0	8.9	[5.6]
Insoluble acids	86.9	85.6	

It was found that 5 grams of the fat obtained by the acetic acid method retained acetic acid equivalent to 1.9 cc. *N* NaOH. Such acidity would have no effect upon iodine absorption nor upon the weight of insoluble acids, and consequently the two samples gave practically identical results for these determinations. The other constants, however, are based upon alkalimetrical principles and acid held by the fat would naturally affect the results. The figures shown in brackets in Table I were obtained by correcting for the amount of acid retained by the fat.

It will be seen that the corrected results vary from those given by the fat separated by the Babcock method only by amounts that can be attributed to experimental error.

It appears, then, that butter fat obtained by the ordinary Babcock method will give normal results for all of the usual determinations, while fat separated in the presence of strong acetic acid gives results apparently abnormal which, however, become normal when we take into account the acidity due to retained acetic acid.

EXPERIMENT II—Hydrochloric acid is also used for the separation of fat from dairy products. Like acetic acid, this also is a volatile acid and might be expected to be retained by the fat, affecting the results as in the case where acetic acid is used.

Eighteen-gram portions of cream were mixed with

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[1] See *Amer. Jour. of Public Health*, **4** (1914), 1009–1015.

[2] Lichtenburg, This Journal, **5** (1913), 786.